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IDENTIFICATION OF SOYBEAN (*Glycine max* (L.) MERRILL) CULTIVARS USING RAPID LABORATORY TECHNIQUES

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Identification of Soybean (*Glycine max* (L.) Merrill) Cultivars Using Rapid Laboratory Techniques[1]

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ABSTRACT

The proliferation of newly developed soybean cultivars has made taxonomic characterization of cultivars via field evaluation of morphological features increasingly inadequate. Because cultivar identification plays an integral role in seed certificaton, the development of laboratory tests which provide improved differentiation has become necessary. Laboratory procedures furnish several additional characteristics useful for genetic purity determination, and offer the promise of rapid and inexpensive analyses for future use in cultivar identification.

In this study several rapid laboratory tests were examined in order to assess their usefulness in characterizing 36 soybean cultivars certified in Ohio. The tests which successfully differentiated the cultivars included hilum color, hypocotyl color, seed coat peroxidase, and electrophoresis of B-amylase and urease in the unimbibed seed. These 5 tests separated the 36 cultivars into 22 groups. Fifteen cultivars were exclusively isolated. Only six cultivars remained in any one group. Acid phosphatase, lactate dehydrogenase, malate dehydrogenase, and glutamic-oxaloacetic transaminase from air-dried seed also were electrophoretically analyzed but did not differentiate any of the cultivars.

More than twice as many cultivars can be isolated using laboratory procedures as compared to field tests currently employed by seed certification agencies. Laboratory testing measures, therefore, offer the potential for greatly enhancing cultivar identification for certification, cultivar review boards, and breeding programs.

Additional index words: electrophoresis, variety, chemotaxonomy, seed, certification.

INTRODUCTION

Cultivar identification serves as the basis for seed certification, which requires that labeling of marketed seed be accurate in order to insure genetic purity. As an example, soybeans sold under the common name Williams should possess the genetic characteristics established by the Plant Variety Protection Office for that cultivar. The proliferation and development of new soybean cultivars stimulated by the passage of the Plant Variety Protection Act in 1970, and the resultant genetic similarities among these cultivars, have made assessment of genetic purity via current methods increasingly difficult.

At present, in order to determine if seed is genetically pure, a certification agency employs inspectors to make field observations of the morphological characteristics of crops grown for seed. Field testing, however, possesses several undesirable characteristics:

- The crop must be grown in areas where the cultivar is well adapted, under the best cultural practices, and during the proper growing season.
- The cultivar must be judged for "trueness-to-type" at precise times.
- An individual who possesses a thorough knowledge of the cultivar is required for identification.
- Field testing generally requires at least 6 months for cultivar determination in order that all characteristics are expressed during a growing season.
- Field testing is expensive, requiring equipment, planting and harvesting personnel, in addition to inspectors and land use.
- The number of morphological characteristics useful in cultivar characterization is no longer adequate for identification of all cultivars.

Current soybean cultivar identification techniques are, for these reasons, inadequate. As a result, the development of laboratory tests to differentiate cultivars recently has been emphasized (40, 51). Laboratory techniques offer the promise of being more rapid and less expensive than field testing. Further, analysis time is flexible and numerous additional traits useful in taxonomic characterizations are available.

The objective of this study was to examine and develop rapid, uncomplicated, inexpensive, and repeatable means of differentiating soybean cultivars which can be applied to seed certification and, in addition, to cultivar reviewing and breeding programs.

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LITERATURE REVIEW

Characteristics which can be employed in the classification of plants into any taxa, including the infraspecific level, are numerous and varied. The most fundamental feature of a plant cultivar is the genetic information housed in DNA which, in turn, is transcribed into an RNA sequence from which a series of amino acids forming a polypeptide chain, or a protein, are translated. Proteins serve as structural components in plant cells, and also as enzymes, catalyzing both primary and secondary metabolic reactions, the products of which are various organic compounds including carbohydrates, lipids, phenolics, etc. These chemical components function in energy storage and cell structure, the latter collectively composing anatomical and subsequent morphological features.

Morphology

Morphological features have been a major criterion in cultivar identification in field testing and, in fact, offer useful information for laboratory identification. Morphological studies of the seed, seedling, and mature crop have demonstrated differences among crop cultivars.

Seed Morphology: Seed morphology has aided cultivar identification within several plant species, although more refined methods in general have prevailed. Baum and Thompson (7) reported that oat cultivars could be identified on the basis of seed size and shape and suggested that observations be automated with the use of scanning devices. Grabe (26), however, pointed out that the use of seed characteristics restricted soybean identification to a limited number of cultivars. In addition, he emphasized that variation of seed shape within a cultivar can occur due to environmental influences on seed development. Payne (51) concurred with this observation and expressed additional concern over the variation of soybean seed size within a cultivar. He also noted that the dull or shiny appearance of a soybean seed coat, previously suggested as an aid in cultivar identification, could be altered in handling.

The color of the soybean hilum has been used extensively in cultivar identification (22, 24, 26, 51), The hilum is the remnant scar of the funiculus which supplies nutrients from the placenta to the developing seed. Hilum color in soybean cultivars (clear, buff, brown, imperfect black, and black) is controlled by the alleles of four genes, some of which are pleiotropic (8). Its ease as a distinguishing characteristic makes the hilum color test a valuable aid in soybean seed certification programs. However, it has been reported that fungal infection as well as the addition of chemical treatments such as fungicides may result in discoloration of the hilum (45), decreasing its use as a stable and distinct cultivar feature.

Few other reliable soybean seed morphological tests for cultivar identification have been reported, limiting the relative usefulness of seed in cultivar differentiation.

Seedling Morphology: Seedling morphology also has been employed in identifying crop cultivars. Hypocotyl length has been proposed for use as a distinguishing feature in soybean cultivars (48). However, within-sample variance exists, especially when specific temperature standards are not maintained. Burris and Knittle (12) demonstrated that excision of cotyledons at 25 vs 30 C caused differing seedling responses. At the higher temperature, the elongation inhibitor of the short cultivars was removed, promoting increased hypocotyl length. In addition, it is important that moisture is maintained at an adequate level throughout the testing period and that hypocotyl length be assessed at the proper time—after 11 days. A premature reading may result in similar hypocotyl length evaluations for all cultivars (11).

The sensitivity of soybean seedlings to the herbicide Metribuzin has been reported to be useful in identifying soybean cultivars (49, 64). But cultural practices, soil type, etc., apparently can influence seedling response to this herbicide, lessening its utility for taxonomic purposes.

The ability of soybean seedlings to germinate under low temperature and saline conditions also has been reported to vary among soybean cultivars. Differences in field emergence among cultivars were demonstrated at 10 C but not at 20 and 30 C (39). A range of 14.8 days was found between the fastest (most cold tolerant) and slowest (least cold tolerant) cultivars tested. Soybean cultivars exhibited differential responses in emergence rate at varying levels of soil salinity. Of the six cultivars studied, two were demonstrated to possess low tolerance to salinity, two intermediate, and two tolerant, based on stand at maturity, necrosis, leaf color, plant height, leaflet width, dry stem weight and seed weight (1). Seed vigor, *i.e.,* the ability of a seed to germinate into a healthy seedling under a wide range of environmental conditions, however, also plays a role in emergence. Because seed vigor may vary among different seed lots within a cultivar, the reliability of emergence tests under certain temperature and saline conditions for use in cultivar identification is lessened considerably (51).

Crop Morphology: Controlled life cycles, *i.e.,* growing plants under greenhouse conditions, also have been used in distinguishing cultivars. Hypocotyl color, flower color, stem pubescence color, leaf shape,

photoperiod, disease resistance, maturity date, and growth habit are characteristics which may vary among soybean cultivars. Hypocotyl pigmentation separates soybean cultivars into at least two groups, purple or green (8). Payne and Morris (47) found four to six pigmentation patterns, depending on testing conditions. The hypocotyl is the seedling organ responsible for cotyledon emergence. Once the soybean seedling has emerged, the hypocotyl exhibits either a green or purple color depending on the cultivar. It has been suggested that hypocotyl pigmentation is the result of the pleiotropic effect of one gene (8).

The purple or green hypocotyl color corresponds to a high or low level of anthocyanin pigment in the seedling. Anthocyanin is a glycoside compound formed by the reaction between a sugar and a cyclic anthocyanidin. It has been demonstrated that environmental conditions which favor an increase in the sugar content of plant tissue, *e.g.,* high light intensity, encourage anthocyanin synthesis in that tissue (42).

Different soybean cultivars also exhibit either gray or brown pubescence, ovate or lanceolate leaf shape, varying maturity dates, and a vertical or branching growth habit (24). Cultivars have varying resistance to certain diseases such as downy mildew, phytophthora root rot, and bacterial blight. Photoperiodic response varies among cultivars, especially those in different maturity groups (16).

The use of controlled life cycles hastens the appearance of these distinguishing features, allowing earlier evaluation relative to field testing. Growing the plants in a greenhouse, however, possesses many of the undesirable characteristics of field testing, *e.g.*, space, time, and expense. In addition, it has been suggested that artificial growing conditions may alter distinguishing plant characteristics (26).

Chemotaxonomic Procedures

A cultivar is a recognizable variant within a species produced in cultivation through hybridization, selection, or other processes (32). Morphological or physiological differences in cultivars are a reflection of the plant's biochemistry which, in turn, is based on the genome. An individual cultivar, then, differs from other cultivars within the species in one or more biochemical characteristics. Procedures which are able to detect qualitative and quantitative biochemical differences among cultivars can be useful in cultivar classification. The use of chemical characteristics to provide taxonomic information is called chemotaxonomy. An array of chemotaxonomic procedures is available for differentiating cultivars. These include spot chemical assays and electrophoretic analysis of proteins.

Spot Assays: Several easily performed spot tests for plant chemicals have been developed for use in cultivar identification. The phenol test (63) is dependent upon a flavonoid reaction in the seed pericarp. It has been demonstrated that this reaction occurs to varying degrees in the epicarp and endocarp of different wheat varieties. This test also has been used in distinguishing bluegrass cultivars (67).

Chemlar and Mostovoj (18) demonstrated that soaking seeds in a solution of potassium hydroxide and hydrochloric acid separated white, yellow, and red wheats.

Rowlands and Corner (53) determined that the seed coats of certain broad bean cultivars exhibited leucoanthocyanin pigment when placed in boiling 2N HCl. Leucoanthocyanin was absent in other broad bean cultivars, making this quick laboratory test useful in broad bean cultivar differentiation.

The peroxidase test, first reported by Buttery and Buzzell in 1968 (13), has been used in separating soybean cultivars into two groups based on the level of peroxidase enzyme located in the seed coat. Inheritance of peroxidase activity in soybean seed coats was shown to be controlled by one gene (17). The presence of a dominant allele results in high activity. Peroxidase levels have likewise been shown to differ among the seeds of barley cultivars (41). The peroxidase staining reaction involves the degradation of H_2O_2 to H_2O and $\frac{1}{2}$ O_2, a reaction catalyzed by peroxidase. The O_2 evolved reacts with guaiacol, producing a dark reddish-brown color. If little or no peroxidase is present, the H_2O_2 will not be degraded appreciably and the solution remains clear.

The presence of fluorescing substances in certain soybean cultivars has been demonstrated (18). Since the fluorescing compounds occur in the soybean root, a 7-day germination period is required. This is undesirable if a more rapid laboratory testing procedure is preferred. In addition, the value of this test in differentiating soybean cultivars is limited as, with few exceptions, most cultivars possess fluorescing substances. The fluorescence test also has been employed in distinguishing between annual and perennial ryegrass, the former producing annuoline, a fluorescing substance in its roots, and the latter possessing no annuoline (33).

An assay useful in determining tannin content in sorghum grain was reported by Price, *et al* (52). Tannin content varied among cultivars and was dependent on grain maturity and drying conditions.

These spot tests are simple and relatively quick to perform. However, seed maturity, age, size, storage, and chemical treatments may influence cultivar evaluation, making their use somewhat limited under laboratory testing conditions (31, 51).

Electrophoresis: The recent development of refined chemotaxonomic procedures useful in cultivar identification has provided unlimited potential. One technique which has emerged as an especially useful tool in analyzing chemical components for taxonomic identification is electrophoresis. Electrophoresis characterizes proteins qualitatively and quantitatively. Because individual plant cultivars often have unique protein electrophoretic patterns, this process often is referred to as "fingerprinting".

Proteins are charged molecules possessing both basic and acidic side chains. This charge allows a protein to move when placed in an electric field. Since every protein has a unique charge and size, each species of protein will migrate to a certain position within an electric field in a given time.

General: Electrophoretic protein analysis has been used extensively for the identification of cultivars within a wide range of plant species. In 1970, Kranski and Bula (34) demonstrated that leaf blade proteins from seven ryegrass cultivars detected by polyacrylamide gel electrophoresis differed quantitatively and qualitatively. Four of the seven cultivars possessed distinct banding patterns. Electrophoretic analysis of peroxidase isozymes from 62 oat cultivars using polyacrylamide gels likewise resulted in several distinct banding patterns (60). Extracts were taken from the above-ground parts of germinated seedlings.

Starch gel electrophoresis also has been shown to be useful in cultivar differentiation. In 1976, Bassiri (4) reported the detection of 3 isozyme systems in shoot extracts from 12 barley cultivars. In combination, esterase, acid phosphatase, and peroxidase isozyme patterns identified all 12 cultivars tested. Extracts from seedling shoots allowed complete identification of 21 wild and cultivated safflower varieties (5). Acid phosphatase and peroxidase analysis were employed in combination to enable these separations. Thirty-six out of 40 broad bean cultivars also were identified by starch gel analysis of young seedling esterase and cathodal peroxidase isozymes (6).

Werner and Sink (65) analyzed leaf extracts from poinsettia cultivars, staining for general protein and peroxidase. Although all 18 cultivars exhibited identical general protein patterns, peroxidase analysis separated the cultivars into 4 groups. In addition, it was demonstrated that when plants of the same cultivar were grown under different temperatures, different electrophoretic banding patterns resulted. The environmental factors under which a plant is germinated, then, may influence protein banding patterns. This suggests the need for strict standardization of growth conditions if germinated or growing plants are analyzed electrophoretically for purposes of cultivar differentiation.

Seed: Air-dried seeds represent a state of suspended metabolic activity in the life cycle of a plant. Gas exchange and respiration rates are low. Messenger RNA activity and subsequent protein synthesis are arrested (43). The electrophoretic analysis of seed proteins, therefore, has become useful in cultivar identification.

In 1971, Bingham and Yeh (9) reported the general protein analysis of 31 alfalfa cultivars. Extracts were made from alfalfa seeds and electrophoresed on polyacrylamide gels. The cultivars tested were separated into four groups based on qualitative and quantitative differences in banding patterns. Similarly, Wrigley and Baxter (68) extracted gliadin proteins from single wheat grains and wheatmeal. Starch gel electrophoresis of the extracts allowed positive identification of a majority of the cultivars tested. In contrast, polyacrylamide gel electrophoresis of gliadin in the seeds of nine wheat cultivars resulted in several banding patterns but did not allow identification of all cultivars (3).

The use of sodium dodecyl sulfate (SDS) in the polyacrylamide slab gel analysis of gliadins extracted from wheat seeds distinguished cultivars not previously differentiated by starch gel methods (56). A similar SDS-polyacrylamide gel system was employed in the analysis of protein extracted from barley seeds (57). The 88 cultivars tested were separated into 29 groups. Position of the grain on the ear, maturity stage, and presence of systemic fungicides were shown to have no effect on the banding patterns.

Two-dimensional polyacrylamide gel electrophoresis of seed polypeptides extracted from peanuts also has been performed (2). The 12 cultivars analyzed exhibited distinct and consistent differences in many of the proteins detected.

Soybean Seed: The electrophoretic analysis of proteins extracted from soybean seeds for cultivar identification purposes was demonstrated first in 1967. Larsen (36) detected the presence of two unique banding patterns from general seed protein electrophoresis, suggesting the use of this characteristic as a supplement to commonly used morphological features in cultivar identification. Characterization of the fast and slow migrating protein bands separated by polyacrylamide electrophoresis was not completed, but the bands were shown to be both distinct and repeatable. Since then, the fast and slow protein bands have been identified as B-amylase (28).

In 1969, Singh, *et al* (58) reported the finding of a genetically controlled variation in soybean trypsin inhibitor (SBTI) among cultivars, as detected by polyacrylamide gel electrophoresis of seed extracts. Clark, *et al* (20) further characterized the two soy-

bean trypsin inhibitor bands detected, establishing the consistent presence of the same SBTI content in all major commercial cultivars of soybeans grown in the United States. SBTI electrophoresis, then, presently has limited value in soybean identification for U. S. cultivars.

Three distinct electrophoretic patterns have been achieved using polyacrylamide gel electrophoresis of oxidative enzymes extracted from the seeds of different soybean cultivars (38). However, since the results of this test coincide with those of Buttery and Buzzell's (13) spot peroxidase test, which is both simpler and more rapid, electrophoretic analysis provides no additional information useful in soybean cultivar identification.

Analysis of urease in soybean seeds also has been shown to be of use in cultivar identification (14). The separation of proteins extracted from the seed with water on polyacrylamide gels resulted in two isozyme patterns useful in categorizing cultivars. Some cultivars exhibited a fast migrating band, others a slow moving band.

The general protein banding patterns of 21 soybean cultivars were found to be identical in a 1977 study (54). Extracts were made from the seeds using phosphate detergent (sodium dodecyl sulfate) and were subjected to polyacrylamide gel electrophoresis.

Gorman and Kiang (25) analyzed 113 commercial soybean cultivars for acid phosphatase, alcohol dehydrogenase, amylase, and tetrazolium oxidase isozymes. Extracts were made from soybean seeds imbibed on moistened paper towels for 6 to 48 hr and applied to a polyacrylamide slab gel. Three different cultivar electrophoretic zymograms were observed for each of the four enzyme systems studied. These tests, in combination, allowed identification of 10 of the 113 cultivars.

Payne and Koszykowski (50) demonstrated a quantitative difference between two banding patterns achieved in the polyacrylamide gel analysis of soybean seed esterase. Although three of the protein bands resolved varied with storage and accelerated aging, one band was consistently present in high or low quantities, corresponding to cultivar differences. Quantitative analyses were made with a densitometer.

Seed and seedling morphology, and chemotaxonomic procedures such as spot tests and the more sophisticated analytical tool, electrophoresis, have emerged as useful methods for cultivar identification. Further chemotaxonomic studies (15, 23, 59, 62), as well as cytological (21, 61, 66) and ultrastructural (19, 35, 44) methods, and ultimately analyses of DNA sequences, offer great potential for reliable identification of cultivars within all plant species.

MATERIALS AND METHODS

Samples Included in Tests

Soybean seed harvested in 1977 and 1978 were requested from institutional growers or from seed companies. Thirty cultivars from the 1977 harvest were received by February 1978. The seed level for each cultivar, *i.e.*, breeder, foundation, registered or certified, is provided in Table 1. Each of the cultivars was tested in the field and the laboratory.

Twenty-nine 1978 cultivars were received by April 1979. Twenty-three of the 30 1977 cultivars were included, bringing the total number of cultivars examined to 36. Table 1 provides the seed level for these cultivars, each of which was tested in the field and laboratory.

All seed samples were stored at room temperature in the laboratory until used.

TABLE 1.—Level of Seed (C-Certified, R-Registered, F-Foundation) from 1977 and 1978 Harvests.

Cultivar	Seed Level	Year(s) of Harvest
Agripro 20	F	1977
Agripro 25	F	1977
Agripro 26	F	1977
Amsoy 71	F/F	1977/1978
Beeson	F/F	1977/1978
Calland	F/F	1977/1978
Cumberland	F	1978
Elf	F	1978
FFR 111	F	1977
FFR 223	F/C	1977/1978
FFR 224	F/C	1977/1978
FFR 335	F/C	1977/1978
FFR 337	C	1978
FFR 444	F/C	1977/1978
Matsoy	C	1977
Mitchell	F/C	1977/1978
Oakland	F	1978
P-61-22	F	1977
Rockford	F	1977
Sloan	F	1978
S 1244	F/F	1977/1978
S 1346	F/F	1977/1978
S 1474	F/F	1977/1978
S 1492	F/F	1977/1978
S 1578	F/F	1977/1978
SRF 150-P	F/R	1977/1978
SRF 200	F/F	1977/1978
SRF 307-P	F/R	1977/1978
SRF 350	F/F	1977/1978
SRF 400	F/R	1977/1978
SRF 450	F/F	1977/1978
Vickery	F	1978
Wayne	F/F	1977/1978
Wells	F/F	1977/1978
Williams	F/F	1977/1978
Woodworth	F/F	1977/1978

Field Tests

In order to verify that the cultivars used in this study were labeled and identified correctly, field tests were performed for seed received both years. The field tests for the 1977 and 1978 cultivars were planted June 2, 1978, and June 5, 1979, respectively. One 3 m long row of each cultivar was planted. Seeds were planted at a rate of six seeds per 30 cm. Border rows were alternated with the rows of cultivars, and two border rows were planted around the perimeter of the plot. Three morphological characteristics were visually observed in the field (stem pubescence color, leaf shape, and flower color) and compared to those outlined by the Ohio Seed Improvement Association (46).

Laboratory Tests

Hilum Color: .A minimum of ten seeds from each cultivar were examined and placed in one of five hilum color categories—clear, buff, brown, imperfect black, and black. (The imperfect black hilum is bordered by a brown line, distinguishing it from the completely black hilum.)

Hypocotyl Color:. In order to determine hypocotyl color, a minimum of ten seeds of each cultivar were placed in a watered medium composed of ⅓ sand, ⅓ soil, and ⅓ vermiculite, and allowed to germinate under fluorescent lighting (450 $\mu Em^{-2}s^{-1}$). After 7 to 10 days, hypocotyl color was examined and the cultivars were placed in one of two categories, green or purple hypocotyl.

Peroxidase Test: The method of Buttery and Buzzell (13) was used to analyze peroxidase content. Seed coats were removed from a minimum of 10 seeds from each cultivar with a razor blade. Each coat then was placed in an individual test tube. Ten drops of 0.5% (v/v) guaiacol were added to each tube. After 10 minutes, one drop of 0.1% (v/v) hydrogen peroxide was added to each tube. Cultivars were placed into one of two groups based on the formation (positive) or absence (negative) of a reddish brown color.

Electrophoresis: The polyacrylamide gel electrophoresis apparatus used in this study included a Buchler Instrument chamber which held 18 electrophoresis tubes and had a total buffer capacity of 1.7 liters. The power supply was ISCO model 493.

Gel preparation: For each electrophoretic run, 18 cylindrical glass tubes 7.5 cm long with an inner diameter of 5 mm were thoroughly washed, dried, inserted into serological stoppers, and placed into the gel stand. Forty ml of 7.5% acrylamide lower gel solution were prepared by mixing 1 part A to 1 part C to 2 parts fresh F (see Appendix). The F solution always was added last. Using a disposable pipette, the gel solution was added to the 6 cm mark on the tubes, making sure that no air bubbles were trapped in the tubes. One drop of distilled water then was added in order to deter formation of a meniscus on the gel's upper surface. The appearance of a sharp boundary line approximately 2 mm below the top of the gel 10 min to 1 hr later indicated that the gel had polymerized. If polymerization did not occur within 1 hr, the gels were placed in a convection oven set at 34 C for 1 hr.

After lower gel polymerization was complete, the liquid on top of the gel was removed with a flick of the wrist and the tubes were placed back into the holder. Forty ml of 2.5% acrylamide upper gel solution were prepared by combining 1 part of B, 2 parts of D, 1 part of E, and 4 parts of distilled water (see Appendix). Six mm of this solution was pipetted onto the top of the lower gel, and 1 drop of distilled water was added to prevent meniscus formation. The gels, in the stand, then were placed under a fluorescent lamp for 30 min, allowing the upper gel to photopolymerize. When ready, the upper gel appeared opaque. Prior to placing the tubes in the electrophoresis apparatus, the liquid on top of the gel was removed with a flick of the wrist. The gels then were used within 3 hr. If not, they were placed in a plastic bag and refrigerated overnight.

The tubes containing gels were placed in the grommets in the upper buffer chamber of the electrophoresis apparatus. Enough tris-glycine buffer (solution G—see Appendix) was added to the lower plastic dish so that the tubes made contact with it. The upper buffer chamber was replaced on the stand and enough tris-glycine buffer was added to easily cover the tops of the tubes. Any air bubbles in the tops of the tubes were removed with a pipette. With a small syringe, 0.05 ml of a concentrated protein extract in a buffer specific for the protein being analyzed was added to the top of each of the gels in the apparatus. The syringe was rinsed with distilled water between sample applications in order to avoid contamination. After all the samples were applied, a drop of tracking dye (0.001% (w/v) bromophenol blue, 10% (w/v) in sucrose) was pipetted onto the top of each gel. Because it is a highly charged, small molecule, the tracking dye migrated faster than the proteins in the sample and therefore monitored the progress of electrophoresis.

The electrodes then were attached, positive polarity to the lower dish, negative to the upper, and the power supply was carefully turned on to 60 mA constant current. This allowed approximately 3.3 mA to run through each of the 18 gels.

After approximately 65 min, the tracking dye reached the bottom of the gel tubes. The power sup-

ply was turned off and the electrodes detached. The tubes were removed from the apparatus. The gels were excised, or rimmed, by inserting a hypodermic needle attached to a water-filled syringe between the gel and the glass tube. The needle was held steady as the tubes were rotated, causing the gel to be detached from the glass tube. The gels then were placed into small glass test tubes, and were stained according to the particular protein being analyzed. In cases where the stain was permanent, the gels were analyzed spectrophotometrically, measuring absorbance at an appropriate wavelength. In this manner, spectrophotometric scans, which represented the banding patterns, were achieved.

Zymograms (pictorial representations of banding patterns) were drawn for each isozyme system analyzed. RF values (RF = distance traveled by protein/distance traveled by tracking dye) also were calculated. Gels were photographed immediately after staining was complete.

Six isozyme systems present in the unimbibed soybean seed were analyzed: B-amylase, acid phosphatase, urease, glutamic-oxaloacetic transaminase, lactate dehydrogenase, and malate dehydrogenase.

Preparation of the seed protein samples

B-amylase, acid phosphatase: An individual sample was prepared by grinding three unimbibed seeds of each cultivar into a fine powder with mortar and pestle set in ice. The powder then was mixed with 6.0 ml of buffer solution which consisted of an equal mixture of 0.1 M sodium acetate and 0.1 N acetic acid made of 10% (w/v) in sucrose (pH 5.0) and maintained at 5 C. The addition of sucrose ensures that the sample will fall to the top of the gel when applied. The soybean material and buffer solution were ground with the pestle. The mortar and pestle were rinsed between extractions in order to avoid sample contamination. The homogenates were placed into centrifuge tubes. If centrifugation was not applied immediately, the tubes were placed in ice. Each of the samples was centrifuged at 20, 200 x g at 5 C for 10 min. The centrifuged samples then were placed in ice until ready for application to the polyacrylamide gel. All samples were prepared fresh daily. A minimum of 15 seeds of each cultivar were analyzed for B-amylase, and a minimum of 3 seeds of each cultivar were analyzed for acid phosphatase.

Urease, glutamic-oxaloacetic transaminase, lactate dehydrogenase, and malate dehydrogenase: An individual sample was prepared by grinding three unimbibed seeds of each cultivar with a mortar and pestle set in ice. The powder then was mixed with 6.0 ml of distilled water made 10% (w/v) in sucrose, and maintained at approximately 5 C. The soybean material and extractant solution were combined with the pestle. Samples were centrifuged as described previously. Again, all samples were prepared daily. A minimum of 15 seeds of each cultivar were analyzed for urease. A minimum of three seeds of each cultivar were tested for glutamic-oxaloacetic transaminase, lactate dehydrogenase, and malate dehydrogenase.

Staining procedures

B-amylase (general protein): After removal from the glass tubes, each gel was soaked for 1 to 8 hr at room temperature in 8 ml of staining solution composed of 0.1 g Coomassie Brilliant Blue in 10 ml of ethanol, combined with 250 ml of 12% (w/v) trichloroacetic acid (3). After the dark blue bands were resolved, the gels were placed in distilled water. Spectrophotometric scans were made at a wavelength of 540 nm.

Acid phosphatase: Each gel was soaked in 8 ml of staining solution for approximately 2 hr at room temperature. The staining solution consisted of 100 ml of pH 4.0, 0.2 M acetate buffer (82 ml 0.2 M acetic acid + 18 ml 0.2 M Na acetate), 100 mg 1-naphthyl phosphate, 100 mg Fast Garnet GBC salt, and 10 drops 10% aqueous $MgCl_2$ (55). After the purple bands were resolved, gels were placed in distilled water. Staining for more than 5 hr resulted in complete gel staining. Spectrophotometric scans were made at a wavelength of 650 nm.

Urease: Each gel was soaked for 10 min at room temperature in 8 ml of staining solution consisting of 25 mg cresol red dissolved in 90 ml of 0.2 M Na acetate buffer (45 ml 0.2 M acetic acid + 45 ml 0.2 M Na acetate) and 60 ml 7% (v/v) acetic acid (14). The gels then were quickly transferred to 8 ml of a solution consisting of 1.6 g urea, 0.1 g Na_2 EDTA, and 25 mg cresol red dissolved in 150 ml distilled water. Within 5 to 20 min, bright purple-red bands were resolved. The banding pattern for urease was recorded immediately after resolution as the solution quickly caused the entire gel to stain. Due to the ephemeral nature of the stain, spectrophotometric scans were not made.

Glutamic-oxaloacetic transaminase: Each gel was soaked for 1 hr at room temperature in a staining solution consisting of 0.53 g L-aspartic acid, 90 mg α—ketoglutaric acid, 50 mg pyridoxal phosphate, 100 ml 0.034 M phosphate solution (0.46 g sodium phosphate monobasic in 100 ml distilled water, plus 1 M NaOH to pH 7.2), and 50 mg fast violet B salt (10). Upon resolution of red bands, the gels were placed in distilled water. Spectrophotometric scans were not made.

Lactate dehydrogenase: Each gel was soaked for 10 min in 8 ml of cold 0.5 M tris-HCl buffer, pH 7.4.

TABLE 2.—Differentiation of 36 Soybean Cultivars Certified in Ohio Based on Five Hilum Color Categories. (Numbers in Parentheses Indicate the Total for Each Group.)

Clear (9)	Buff (2)	Brown (5)	Imperfect Black (6)	Black (14)
Amsoy 71	Agripro 20	Mitchell	Agripro 25	Calland
FFR 111	S 1492	Sloan	Agripro 26	Elf
FFR 223		S 1474	Beeson	FFR 224
Matsoy		S 1578	Cumberland	FFR 335
P-61-22		SRF 307-P	Rockford	FFR 337
S 1346			Wells	Oakland
SRF 150				S 1244
SRF 200				SRF 350
Vickery				SRF 400
				SRF 450
				Wayne
				Williams
				Woodworth
				FFR 444

The gel then was transferred to a staining solution consisting of 8 ml Na lactate, 40 mg nicotine adenine dinucleotide, 8 ml 0.1 M NaCl, 8 ml 0.005 M $MgCl_2$, 20 ml 0.5 M tris-HCl buffer (pH 7.4), 20 ml of 0.1% (w/v) nitro blue tetrazolium solution, and 2 ml 0.1% (w/v) phenyl methyl sulfate solution, (10). Staining occurred at room temperature. After 1½ hr, purple bands were resolved and the gels were placed in distilled water. Spectrophotometric scans were made at a wavelength of 650 nm.

TABLE 3.—Differentiation of 36 Soybean Cultivars Certified in Ohio Based on Hypocotyl Color. (Numbers in Parentheses Indicate the Total for Each Group.)

Hypocotyl Color	
Purple (27)	Green (9)
Agripro 20	FFR 335
Agripro 25	FFR 337
Agripro 26	Sloan
Amsoy 71	S 1492
Beeson	SRF 307-P
Calland	SRF 350
Cumberland	Wayne
Elf	Williams
FFR 111	Woodworth
FFR 223	
FFR 224	
Matsoy	
Mitchell	
Oakland	
P-61-22	
Rockford	
S 1244	
S 1346	
S 1474	
S 1578	
SRF 150	
SRF 200	
SRF 400	
SRF 450	
Vickery	
Wells	
FFR 444	

Malate dehydrogenase: Each gel was soaked at room temperature for 1 hr in 8 ml of staining solution consisting of 25 ml of 0.2 M tris-HCl buffer (pH 8.0), 25 ml distilled water, 0.5 g L-malic acid, 0.2 ml of 0.1 M potassium cyanide, 0.01 M nicotine adenine dinucleotide, 8 mg phenyl methyl sulfate, and 25 mg nitro blue tetrazolium (55). After resolution of purple bands, gels were removed and placed in distilled water. Spectrophotometric scans were made at a wavelength of 650 nm.

RESULTS

Field Tests

Results of the field tests were consistent with cultivar characteristics outlined by the Ohio Seed Improvement Association, with two exceptions. Agripro 20, which was listed as possessing a brown hilum, was placed in the buff category in this study, as well as in another report (51). In addition, SRF 307-P, listed as having a buff hilum, was placed in the brown grouping (30).

Laboratory Tests

Hilum Color: Hilum color determinations categorized the Ohio soybean cultivars as indicated in Table 2. The 36 soybean cultivars were subdivided into 5 groups. The largest group (black) contained 14 cultivars. Results for the same cultivar from the two different growing seasons were identical.

Hypocotyl Color: Hypocotyl color determination categorized the 36 Ohio soybean cultivars as indicated in Table 3. A total of 27 cultivars were classified as purple and 9 cultivars possessed green

hypocotyls. A correlation between the seedling hypocotyl color and the flower color was shown to exist (8). Cultivars possessing green hypocotyls produced white flowers while cultivars having purple hypocotyls produced purple flowers. Results for the same cultivar from the two different growing seasons were identical. The genetic linkage between imperfect black hila and purple hypocotyl color, demonstrated in past work, was substantiated.

Peroxidase Test: The peroxidase test grouped the 36 cultivars into 2 categories as indicated in Table 4. Sixteen cultivars yielded a positive peroxidase reaction; 20 cultivars demonstrated a negative reaction. Results for the same cultivar from the two different growing seasons were the same.

Electrophoresis

B-amylase: Two patterns resulted when gels were stained for general protein. One exhibited a fast moving B-amylase band (Rf=0.51), the other a slow moving band (Rf=0.46), as indicated by Figure 1.

The 36 cultivars were categorized as possessing the fast or slow moving B-amylase band as indicated in Table 5. Twenty-eight cultivars possessed a fast moving B band; eight cultivars demonstrated a slow moving A band. Banding patterns within the same cultivar did not differ among growing seasons. As has been reported previously (51), cultivars with black, brown, or buff hila exhibit the slow B-amylase band,

TABLE 4.—Differentiation of 36 Soybean Cultivars Certified in Ohio Based on Positive or Negative Seed Coat Peroxidase Reaction. (Numbers in Parentheses Indicate the Total for Each Group.)

Peroxidase Test	
Positive (16)	**Negative (20)**
Agripro 20	Agripro 25
Amsoy 71	Agripro 26
Cumberland	Beeson
FFR 111	Calland
FFR 223	Elf
Matsoy	FFR 224
Mitchell	FFR 335
P-61-22	FFR 337
Rockford	Oakland
S 1244	Sloan
S 1578	S 1346
SRF 150	S 1474
SRF 200	S 1492
SRF 450	SRF 307-P
Vickery	SRF 350
Williams	SRF 400
	Wayne
	Wells
	Woodworth
	FFR 444

TABLE 5.—Differentiation of 36 Soybean Cultivars Certified in Ohio Based on Electrophoretic Analysis of B-Amylase Extracted from the Unimbibed Seeds. (Numbers in Parentheses Indicate the Total for Each Group.)

B-Amylase	
Slow (8)	**Fast (28)**
Agripro 20	Agripro 25
Amsoy 71	Agripro 26
Beeson	Calland
FFR 111	Cumberland
FFR 223	Elf
Rockford	FFR 224
SRF 200	FFR 335
Wells	FFR 337
	Matsoy
	Mitchell
	Oakland
	P-61-22
	Sloan
	S 1244
	S 1346
	S 1474
	S 1492
	S 1578
	SRF 150
	SRF 307-P
	SRF 350
	SRF 400
	SRF 450
	Vickery
	Wayne
	Williams
	Woodworth
	FFR 444

TABLE 6.—Differentiation of 36 Soybean Cultivars Certified in Ohio Based on Electrophoretic Analyses of Urease Extracted from the Unimbibed Seeds. (Numbers in Parentheses Indicate the Total for Each Group.)

Urease		
One (17)	**Two (16)**	**Both (3)**
Calland	Agripro 20	Agripro 26
Cumberland	Agripro 25	Beeson
Elf	Amsoy 71	FFR 335
FFR 224	FFR 111	
FFR 337	FFR 223	
Oakland	Matsoy	
S 1244	Mitchell	
S 1492	P-61-22	
SRF 150	Rockford	
SRF 307-P	Sloan	
SRF 350	S 1346	
SRF 400	S 1474	
Wayne	S 1578	
Wells	SRF 200	
Williams	SRF 450	
Woodworth	Vickery	
FFR 444		

while cultivars with clear or imperfect black hila possess either the slow or fast B-amylase band. This limits the usefulness of the test somewhat.

Urease: Two isozyme banding patterns resulted when gels were stained for urease. One exhibited two bands (Rf=0.11, 0.30), the other one band (Rf =0.30), as indicated by Figure 2. Spectrophotometric scans were attempted but did not succeed due to the ephemeral nature of the urease stain

The 36 cultivars were categorized as possessing the 1 or 2 band(s), as indicated in Table 6. Three cultivars exhibited either banding pattern, *i.e.*, seed urease varied. Since the seed used was deemed pure, these cultivars apparently possessed the genetic ability to produce either urease banding pattern. Seventeen cultivars possessed 2 bands, 16 cultivars demonstrated 1 band and 3 cultivars exhibited both banding patterns Banding patterns did not differ within the same cultivar from different growing seasons.

Malate dehydrogenase, lactate dehydrogenase, glutamic-oxaloacetic transaminase, and acid phosphatase: Analysis of malate dehydrogenase resulted in

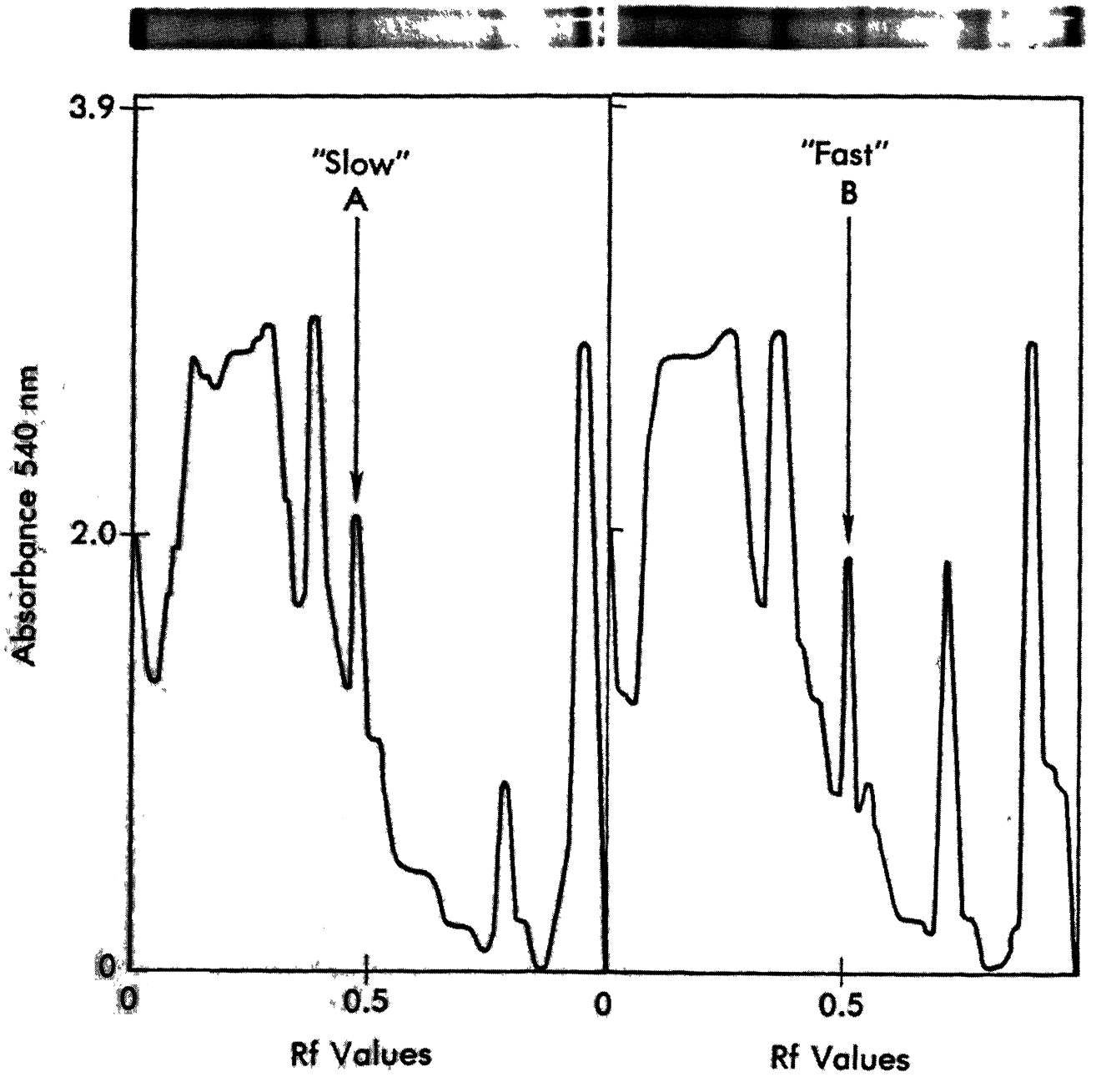

FIG. 1.—Spectrophotometric scans and photographs of the two B-amylase banding patterns of soybean cultivars, slow (A) and fast (B).

identical banding patterns for all cultivars studied One primary band (Rf=0.47) was resolved after 1 hr of staining (Fig 3). Other less intense bands became evident when the gels were stained for a longer period of time All cultivars exhibited identical lactate dehydrogenase isozymes after 1½ hr. The major band had an Rf value of 0.23 (Fig 3). Again, prolonged staining resulted in the appearance of several less intense bands. Glutamic-oxaloacetic transaminase electrophoresis resulted in the same banding pattern for all cultivars After 1 hr of staining, two bands were evident (Rf=0.29 and 0.49) (Fig. 3). Finally, acid phosphatase isozymes were the same for all cultivars. After 2 hr, one primary band (Rf=0 49) was resolved (Fig. 3). A diffusely stained area between Rf 0.33 and 0.4 appeared above this main band upon prolonged staining Thus, electrophoresis of malate dehydrogenase, lactate dehydrogenase, glutamic-oxaloacetic transaminase, and acid phosphatase failed to resolve differences in banding patterns for any of the soybean cultivars examined. Results for the six isozyme systems studied did not differ when the same cultivar from the two different growing seasons was analyzed.

Summary of Results

Combining the data from the five tests successful in differentiating soybean cultivars in this study culminated in the separation depicted in Figure 4. Of the original 36 cultivars examined, 15 cultivars can be identified using the 5 tests in this identification system. Further, 22 groupings were established, with no grouping possessing more than 6 cultivars. Appendix Table II provides a key for the 36 cultivars studied.

DISCUSSION

Laboratory Tests

A test useful for cultivar identification should possess several characteristics. It should be relatively uncomplicated, quick, consistent, and inexpensive to perform. The test also should allow immediate observation of "off-types." Finally, if possible, the feature under examination should be possessed by individual plants or plant parts. Cultivars of self-pollinated crops such as soybeans exhibit homogeneous characteristics, and thus individual plant parts are useful in cultivar determination (26).

Since it is unlikely that any single test will completely separate all cultivars, several tests were examined in this study and the results incorporated into a key (see Appendix Table II). Unimbibed seeds were used for all electrophoretic protein determinations because they are relatively stable physiologically, and therefore were expected to provide repeatable results under standardized conditions. Further, in

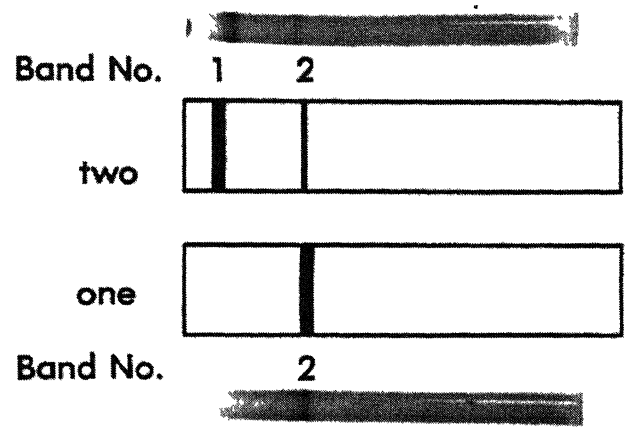

FIG. 2.—Zymograms and photographs of the two urease banding patterns of soybean cultivars, two and one.

general, tests which yielded qualitative results, *e.g.*, type of isozyme present, type of pigment present, etc., were employed, because qualitative data are less subject to such factors as seed vigor, storage, etc, than quantitative parameters. Finally, testing seeds of the same cultivar from different years allowed a comparison of results from different seed lots, addressing the question of repeatability between growing seasons.

The hilum color test was useful but possessed several undesirable characteristics. Treated seed masked the hilum such that categorization was difficult. In addition, the difference between a buff and a brown hilum was minimal and differentiating the two was difficult. Given untreated, healthy seed, and experience, the hilum color test is, however, a valuable technique for distinguishing cultivars.

The hypocotyl color test, although requiring a minimum of 7 days to perform, proved to be a reliable method for separating soybean cultivars. However, the separation resulted in only two groupings, with a large majority of cultivars falling into the purple hypocotyl category (Table 3). Still, because of the association between flower color and hypocotyl color, this test is much faster than field observation.

The peroxidase test emerged as a useful assay for cultivar identification purposes. Results obtained were consistent, but only when great care was taken to remove and test only the seed coat and none of the cotyledonary tissue. The latter yields a positive peroxidase test regardless of the seed coat reaction.

Electrophoresis proved to be an excellent means of characterizing variations in protein content among soybean cultivars. The relatively low equipment cost (approximately $1,500), swiftness of analysis, ease of operation, and ability to analyze seed protein

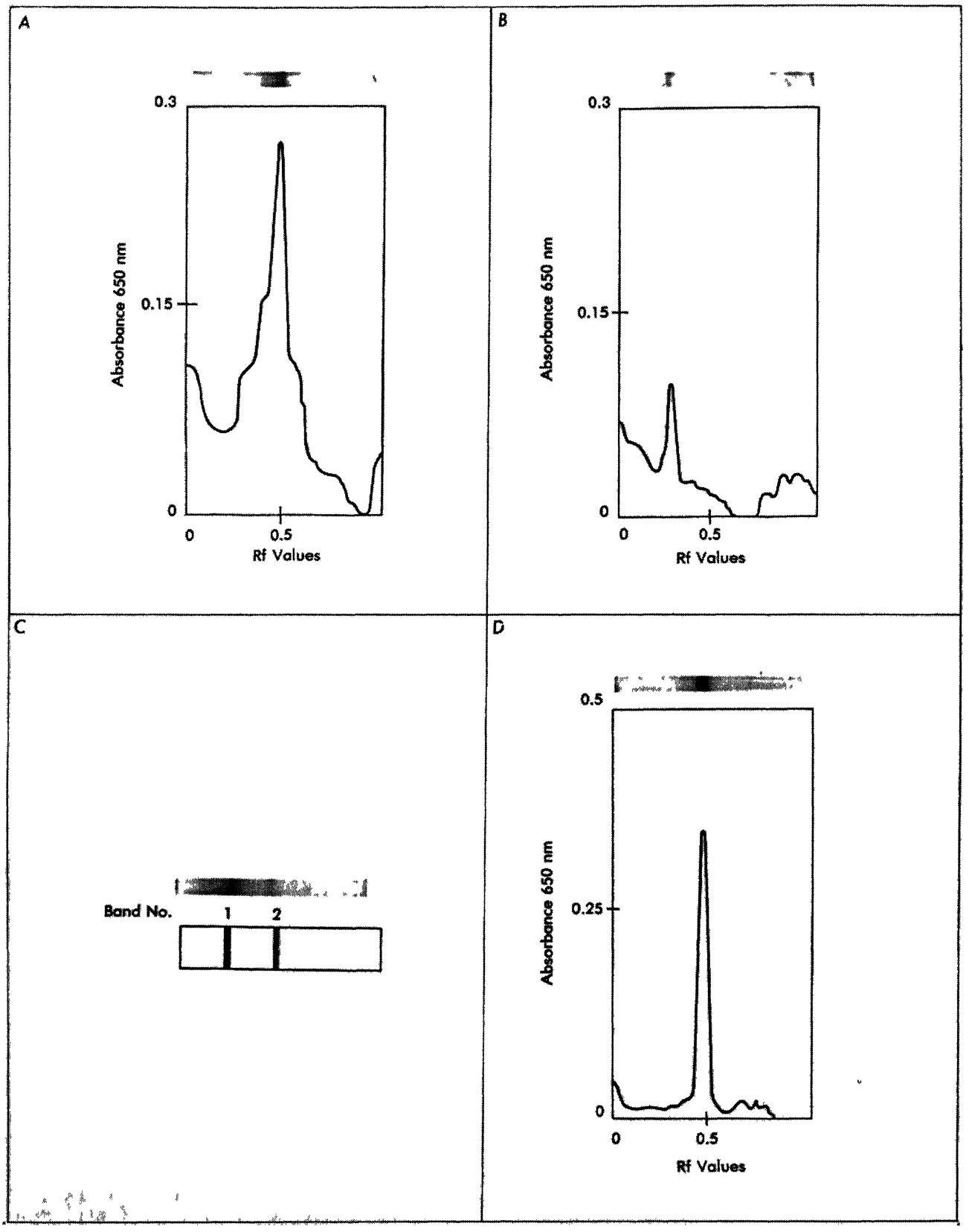

FIG. 3.—Spectrophotometric scan, photograph, and zymogram of malate dehydrogenase (A), lactate dehydrogenase (B), glutamic-oxaloacetic transaminase (C), and acid phosphatase (D) banding patterns exhibited by soybean cultivars.

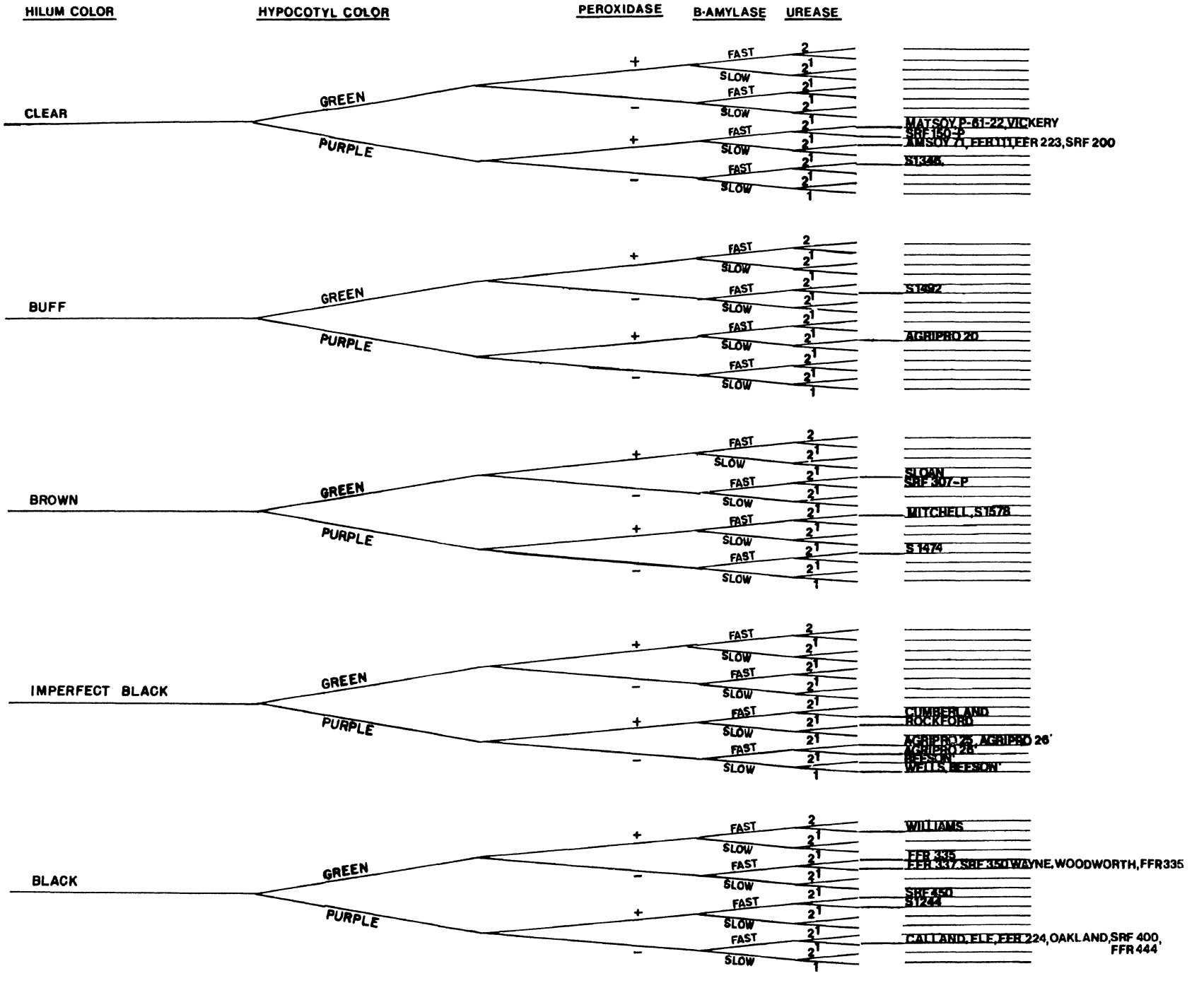

FIG. 4.—Schematic diagram illustrating the separation of 36 soybean cultivars via laboratory tests.

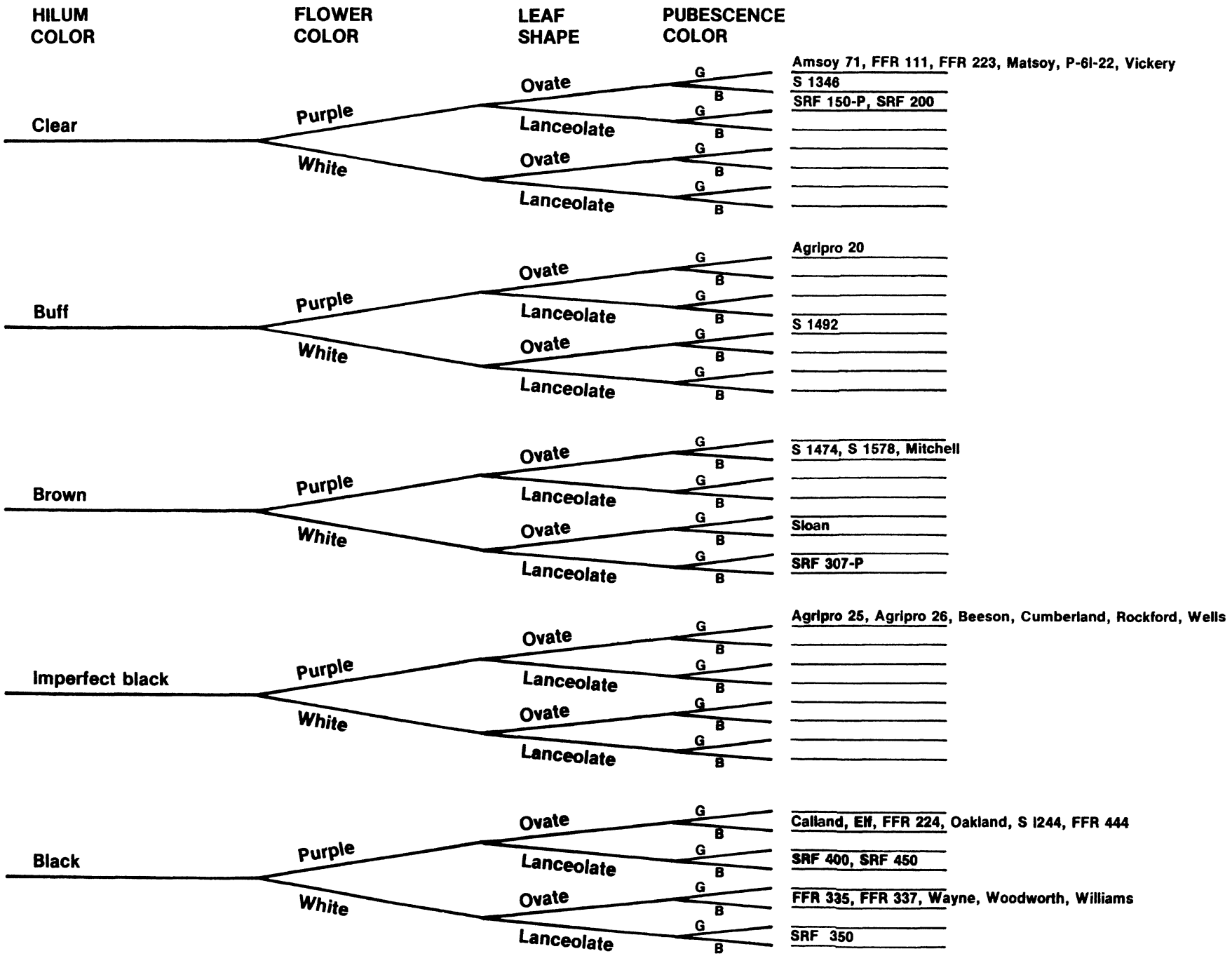

FIG. 5.—Schematic diagram illustrating the separation of 35 soybean cultivars via field tests.

samples repeatably contributed to the feasibility of its use for cultivar identification.

The unimbibed soybean seed was shown to possess at least six isoyzme systems: B-amylase, acid phosphatase, urease, glutamic-oxaloacetic transaminase, lactate dehydrogenase, and malate dehydrogenase. Variations among cultivars were detected for two isozyme systems, B-amylase and urease. In past work (8, 37), the B-amylase band was shown to be controlled by two codominant alleles at a single locus.

The banding pattern achieved for soybean seed urease labeled "two" in this study differed from that reported originally by Buttery and Buzzell (14). The former study resulted in two banding patterns, one with a fast moving band, another possessing a band of slower mobility. The urease banding pattern was shown to be monogenically controlled, the fast migrating band dominant over the slow moving band (14). In the present study, two bands were resolved for cultivars deemed by Buttery and Buzzell as possessing the slow moving band. Similar results have been reported in other studies (51). It is possible that the slow moving band may dissociate due to factors such as the buffer employed, stability of the molecule, etc., producing a faster moving artifact (14).

The protein patterns detected for the four other isozyme systems analyzed (acid phosphatase, glutamic-oxaloacetic transaminase, lactate dehydrogenase, and malate dehydrogenase) under the described conditions, failed to assist in separating the cultivars studied. Recent work using different analytical conditions (25, 27), *i.e.,* gel and buffer pH, indicated that electrophoresis of acid phosphatase yielded varying protein banding patterns among cultivars. Applying the gel and buffer conditions employed in that study to the electrophoresis of these two isozyme systems in the unimbibed seed might enable further separation of cultivars. The application of numerous electrophoretic systems employing various gel and buffer types to the large number of enzymes present in the soybean seed provides endless possibilities for cultivar characterization.

The separation of the cultivars using the laboratory tests demonstrated in this study shows considerable improvement over the separation of the same cultivars achieved via field testing. As indicated in Figure 5, the use of hilum color, flower color, leaf shape, and stem pubescence color, the 4 most commonly employed characteristics in field testing, separates the 36 cultivars into 13 groups. Only six cultivars are isolated exclusively. The largest number of cultivars remaining in any one group is six. Laboratory testing is therefore a more effective means of differentiating cultivars in terms of the number of cultivars which may be exclusively identified.

Feasibility of Laboratory Testing Procedures

Laboratory tests offer several important advantages over field testing in terms of effectiveness of identification, distinct and readily observable characteristics, and use of space. Further, it is important to note that the number of distinguishing characteristics, especially of a chemical nature, which potentially can be detected in laboratory analysis far exceeds the number of morphological features useful in differentiating cultivars via field testing.

There are, however, many unanswered questions regarding the use of laboratory tests for seed certification purposes. The laboratory testing format must be feasible in terms of time, cost, and logistical considerations. At present, a sequential sampling method developed by the Association of Official Seed Certification Agencies is employed in field testing (29). In order for a field to be certified, a minimum of 3,000 plants are observed in a random fashion. If eight or less off-types are observed, the field is accepted. If 31 or more off-types are detected, the field is rejected. Additional plants are observed if between 9 and 30 off-types are found. A final decision regarding certification is made after a minimum of 10,000 plants are observed. At this point, if 62 or more off-types have been detected, the field may not be certified.

The cost of certification to the seedman averages $2.40/acre (29). If the yield per acre is 50 bushels, the cost is 5¢/bushel. Including the 1¢ charge for each certification tag, the total cost for certification approaches 6¢/bushel.

Assuming identical sample size, laboratory testing procedures cannot presently compete with field testing in terms of cost and time. Although the material cost is low, the number of samples which can be analyzed in a reasonable amount of time, especially for electrophoretic analysis, is small, requiring a large number of tests and excessive time. However, as more and more cultivars of increasing homogeneity are developed, field testing procedures will undoubtedly become less adequate, encouraging technological improvement of laboratory tests. In addition, development of a sampling method which would require a smaller representative sample size would enhance the use of laboratory techniques in the routine analysis of seed for certification purposes.

Laboratory testing can be applied to other facets of seed certification programs. Some certification agencies augment initial field inspection with laboratory procedures such as hypocotyl color and the peroxidase test, especially if a problem with off-types is detected during field inspection. Further testing procedures such as electrophoretic analysis expand the number of tests useful for this purpose.

Certification agences also may field test seed from the previous year's harvest for purposes of establishing cultivar purity. This is especially useful in determining if a certain lot of breeder, foundation, or registered seed is adequately pure for further seed multiplication. In such a test, approximately 100 seeds are tested, a number which could be analyzed reasonably via laboratory procedures.

Laboratory cultivar identification not only benefits seed certification, but crop breeding and cultivar reviewing programs as well. The crop breeder is interested in developing new crop cultivars. Laboratory tests offer a quick method of screening new plant genotypes. In addition, these tests provide a feasible manner of detecting any changes in the genome of a seed stock as it is multiplied or stored.

Cultivar review boards are composed of members of the American Seed Trade Association, Association of Official Seed Certifying Agencies, Crop Science Society of America, National Council of Commercial Plant Breeders, U. S. Dept. of Agriculture, and the Agricultural Research Service, USDA. Review boards serve to examine and evaluate requests for certification of new cultivars developed by breeders in industry and public agencies. These boards must ensure that each new crop cutivar is unique in one or more genetically based traits. Laboratory tests offer an essentially endless source of information which characterizes cultivars. This becomes increasingly important as more and more cultivars are developed.

CONCLUSIONS

- Five laboratory tests—hilum color, hypocotyl color, seed coat peroxidase, B-amylase and urease electrophoresis—successfully separated 36 soybean cultivars into 22 groups. Fifteen cultivars were isolated exclusively. The largest number of cultivars remaining in any one group was six.
- The laboratory testing procedures provided improved separation of the 36 cultivars over field testing methods currently employed by seed certification agencies.
- The laboratory tests are not at present competitive with field testing for seed certification programs in terms of cost and time of analysis. However, technological improvement of laboratory tests, modification of sampling procedures presently used, as well as the proliferation of increasingly homogeneous soybean cultivars, magnify the importance of incorporating laboratory testing procedures into future certification programs.
- Laboratory tests useful in differentiating soybean cultivars can benefit crop breeders and cultivar review boards, and supplement current seed certification programs.

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APPENDIX TABLE I.—Procedure for Preparing 7.5% Acrylamide Lower Gel, pH 8.9; 2.5% Acrylamide Upper Gel, pH 6.9; and Tris Glycine Buffer, pH 8.3.

Solutions					
Lower Gel			**Upper Gel**		
A. 1M HCL	24.0	ml	B. 1M H_3PO	25.6	ml
Tris	18.2	g	Tris	5.7	g
TEMED*	0.23	ml	TEMED*	0.46	ml
Water to 100 ml (ph 8.9)			Water to 100 ml (pH 6.9)		
C. Acrylamide	30.0	g	D. Acrylamide	10.0	g
Bisacrylamide	0.8	g	Bisacrylamide	2.5	g
Water to 100 ml			Water to 100 ml		
F. Ammonium persulfate	0.14	g	E. Riboflavin	4	mg
Water to 100 ml			Water to 100 ml		
G. Buffer (pH 8.3)					
Tris	3.0	g			
Glycine	14.4	g			
Water to 100 ml					

*TEMED is N, N, N', N' tetramethylethylenediamine

APPENDIX TABLE II.—Key Useful in Identifying 36 Soybean Cultivars Certified in Ohio.

- Hilum Clear
  - Peroxidase Positive
    - B-Amylase Fast
      - Urease Two—Matsoy, P-61-22, Vickery
      - Urease One—SRF 150
    - B-Amylase Slow—Amsoy 71, FFR 111, FFR 223, SRF 200
  - Peroxidase Negative—S 1346
- Hilum Not Clear
  - Hilum Buff
    - Hypocotyl Green—S 1492
    - Hypocotyl Purple—Agripro 20
- Hilum Not Buff
  - Hilum Brown
    - Hypocotyl Purple
      - Peroxidase Positive—Mitchell, S 1578
      - Peroxidase Negative—S 1474
    - Hypocotyl Green
      - Urease 2—Sloan
      - Urease 1—SRF 307-P
- Hilum Not Brown
  - Hilum Imperfect Black
    - Peroxidase Positive
      - B-Amylase Fast—Cumberland
      - B-Amylase Slow—Rockford
    - Peroxidase Negative
      - B-Amylase Fast
        - Urease 2—Agripro 25, Agripro 26
        - Urease 1—Agripro 26
      - B-Amylase Slow
        - Urease 2—Beeson
        - Urease 1—Wells, Beeson
- Hilum Not Imperfect Black
  - Hypocotyl Purple
    - Peroxidase Positive
      - Urease 2—SRF 450
      - Urease 1—S 1244
    - Peroxidase Negative—Calland, Elf, FFR 224, Oakland, SRF 400, FFR 444
    - Hypocotyl Green
      - Peroxidase Positive—Williams
      - Peroxidase Negative
        - Urease 2—FFR 335
        - Urease 1—FFR 337, SRF 350, Wayne, Woodworth, FFR 335

BETTER LIVING IS THE PRODUCT

of research at the Ohio Agricultural Research and Development Center. All Ohioans benefit from this product.

Ohio's farm families benefit from the results of agricultural research translated into increased earnings and improved living conditions. So do the families of the thousands of workers employed in the firms making up the state's agribusiness complex.

But the greatest benefits of agricultural research flow to the millions of Ohio consumers. They enjoy the end products of agricultural science—the world's most wholesome and nutritious food, attractive lawns, beautiful ornamental plants, and hundreds of consumer products containing ingredients originating on the farm, in the greenhouse and nursery, or in the forest.

The Ohio Agricultural Experiment Station, as the Center was called for 83 years, was established at The Ohio State University, Columbus, in 1882. Ten years later, the Station was moved to its present location in Wayne County. In 1965, the Ohio General Assembly passed legislation changing the name to Ohio Agricultural Research and Development Center—a name which more accurately reflects the nature and scope of the Center's research program today.

Research at OARDC deals with the improvement of all agricultural production and marketing practices. It is concerned with the development of an agricultural product from germination of a seed or development of an embryo through to the consumer's dinner table. It is directed at improved human nutrition, family and child development, home management, and all other aspects of family life. It is geared to enhancing and preserving the quality of our environment.

Individuals and groups are welcome to visit the OARDC, to enjoy the attractive buildings, grounds, and arboretum, and to observe first hand research aimed at the goal of Better Living for All Ohioans!

The State Is the Campus for Agricultural Research and Development

Ohio's major soil types and climatic conditions are represented at the Research Center's 12 locations.

Research is conducted by 15 departments on more than 7000 acres at Center headquarters in Wooster, eight branches, Pomerene Forest Laboratory, North Appalachian Experimental Watershed, and The Ohio State University.

Center Headquarters, Wooster, Wayne County: 1953 acres

Eastern Ohio Resource Development Center, Caldwell, Noble County: 2053 acres

Jackson Branch, Jackson, Jackson County: 502 acres

Mahoning County Farm, Canfield: 275 acres

Muck Crops Branch, Willard, Huron County: 15 acres

North Appalachian Experimental Watershed, Coshocton, Coshocton County: 1047 acres (Cooperative with Science and Education Administration/Agricultural Research, U. S. Dept. of Agriculture)

Northwestern Branch, Hoytville, Wood County: 247 acres

Pomerene Forest Laboratory, Coshocton County: 227 acres

Southern Branch, Ripley, Brown County: 275 acres

Vegetable Crops Branch, Fremont, Sandusky County: 105 acres

Western Branch, South Charleston, Clark County: 428 acres